

Applicants: Short and Keller
Application No.: 09/848,095
Filed: May 3, 2001
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In the Claims

Please amend claims 1, 13, 26, 39, 46, 54, and 55 to read as follows:

1. (Currently Amended) A method for identifying bioactivities or biomolecules using high throughput screening of nucleic acid comprising:
 - a) providing a an environmental gene library containing a plurality of clones, wherein DNA for generating the library is naturally occurring and obtained from ~~more than one~~ a mixed population of uncultured organisms;
 - b) encapsulating a bioactive substrate and at least one clone of the library in a gel microdroplet, wherein an bioactivity or biomolecule produced by the clone is detectable by a ~~difference~~ change in fluorescence of the substrate prior to contacting with the at least one clone as compared to after the contacting; and
 - c) screening the microdroplet with an assay or an analyzer that detects the presence therein of the change in the substrate, wherein the change indicates the identity of the bioactivity or biomolecule.
2. (Previously Amended) The method of claim 1, wherein the bioactivity is provided by an enzyme that is selected from the group consisting of lipases, esterases, proteases, glycosidases, glycosyl transferases, phosphatases, kinases, mono- and dioxygenases, haloperoxidases, lignin peroxidases, diarylpropane peroxidases, epoxide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases, and acylases.

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3. (Original) The method of claim 1, wherein the library is generated in a prokaryotic cell.
4. (Original) The method of claim 1, wherein the library is generated in a *Streptomyces* sp.
5. (Previously Amended) The method of claim 4, wherein the *Streptomyces* sp. is *Streptomyces venezuelae*.
6. (Original) The method of claim 3, wherein the prokaryotic cell is gram negative.
7. (Original) The method of claim 1, wherein the gene library is an expression library.
8. (Previously Amended) The method of claim 7, wherein the expression library contains DNA obtained from extremophiles.
9. (Original) The method of claim 8, wherein the extremophiles are thermophiles.
10. (Previously Amended) The method of claim 9, wherein the extremophiles are selected from the group consisting of hyperthermophiles, psychrophiles, halophiles, psychrotrophs, alkalophiles, and acidophiles.
11. (Original) The method of claim 1, wherein the bioactive substrate comprises C12FDG.
12. (Original) The method of claim 1, wherein the bioactive substrate comprises a lipophilic tail.

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13. (Currently Amended) The method of claim 1, wherein ~~samples~~ the clones are heated before step b).
14. (Original) The method of claim 13, wherein the heating is at about 70°C.
15. (Previously Amended) The method of claim 14, wherein the heating occurs for about 30 minutes.
16. (Original) The method of claim 1, wherein the analyzer comprises a fluorescent analyzer.
17. (Original) The method of claim 16, wherein the fluorescent analyzer is a FACS apparatus.
18. (Original) The method of claim 1, wherein the library is biopanned before step b).
19. (Previously Amended) The method of claim 3, wherein the prokaryotic cell is *E. coli*.
20. (Previously Amended) The method of claim 19, wherein prior to step b), the library in *E. coli* is transferred to a *Streptomyces* sp.
21. (Original) The method of claim 20, wherein the *Streptomyces* sp. is *Streptomyces venezuelae*.

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22. (Original) The method of claim 1, wherein the library is normalized before step b).
23. (Original) The method of claim 1, further comprising co-encapsulating an indicator cell in step b).
24. (Original) The method of claim 1, wherein the analyzer is a chromogenic analyzer.
25. (Original) The method of claim 1, wherein the assay is an immunoassay.
26. (Currently Amended) A method for identifying bioactivities or biomolecules using high throughput screening of nucleic acid comprising:
 - a) providing a an environmental gene library containing a plurality of clones, wherein the nucleic acid for generating the library is naturally occurring and obtained from ~~more than one~~ a mixed population of organisms;
 - b) inserting a ~~bioactive substrate~~ polynucleotide into the clones of the library, wherein the polynucleotide encodes a bioactive protein substrate, wherein a fluorescence change in the substrate is detectable in the presence of a bioactivity or biomolecule; and
 - c) screening the clones from the normalized library with an assay or an analyzer that detects the presence therein of the fluorescence change in the substrate, wherein the fluorescence change in the substrate identifies the bioactivity or biomolecule.

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27. (Previously Amended) The method of claim 26, further comprising encapsulating the clone and the bioactive substrate prior to screening.
28. (Previously Amended) The method of claim 27, wherein the bioactivity is provided by an enzyme that is selected from the group consisting of lipases, esterases, proteases, glycosidases, glycosyl transferases, phosphatases, kinases, mono- and dioxygenases, haloperoxidases, lignin peroxidases, diarylpropane peroxidases, epoxide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases, and acylases.
29. (Original) The method of claim 27, wherein the library is generated in a prokaryotic cell.
30. (Original) The method of claim 27, wherein the library is generated in a *Streptomyces* sp.
31. (Previously Amended) The method of claim 30, wherein the *Streptomyces* sp. is *Streptomyces venezuelae*.
32. (Original) The method of claim 29, wherein the prokaryotic cell is gram negative.
33. (Original) The method of claim 27, wherein the gene library is an expression library.
34. (Previously Amended) The method of claim 33, wherein the expression library contains DNA obtained from extremophiles.
35. (Original) The method of claim 34, wherein the extremophiles are thermophiles.

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36. (Previously Amended) The method of claim 35, wherein the extremophiles are selected from the group consisting of hyperthermophiles, psychrophiles, halophiles, psychrotrophs, alkalophiles, and acidophiles.
37. (Original) The method of claim 27, wherein the bioactive substrate comprises C12FDG.
38. (Original) The method of claim 27, wherein the bioactive substrate comprises a lipophilic tail.
39. (Currently Amended) The method of claim 27, wherein ~~samples~~ the clones are heated before step b).
40. (Original) The method of claim 39, wherein the heating is at about 70°C.
41. (Previously Amended) The method of claim 40, wherein the heating occurs for about 30 minutes.
42. (Original) The method of claim 27, wherein the analyzer comprises a fluorescent analyzer.
43. (Original) The method of claim 42, wherein the fluorescent analyzer is a FACS apparatus.

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44. (Original) The method of claim 27, wherein the library is biopanned before step b).
45. (Original) The method of claim 29, wherein the prokaryotic cell is *E. coli*.
46. (Currently Amended) The method of claim 45, wherein prior to step b), the library in *E. coli* is transferred to a myceliate bacteria or fungi.
47. (Original) The method of claim 46, wherein the myceliate fungi is an *Actinomyces* sp.
48. (Original) The method of claim 46, wherein the myceliate bacteria is a *Streptomyces* sp.
49. (Original) The method of claim 47, wherein the *Streptomyces* sp. is *Streptomyces venezuelae*.
50. (Original) The method of claim 27, wherein the library is normalized before step b).
51. (Original) The method of claim 27, further comprising co-encapsulating an indicator cell in step b).
52. (Original) The method of claim 27, wherein the analyzer is a chromogenic analyzer.
53. (Original) The method of claim 27, wherein the assay is an immunoassay.

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54. (Currently Amended) The method of claim 26, wherein the bioactive substrate is a ~~polynucleotide encoding a fusion protein comprising the~~ a protein substrate flanked by two fluorescent proteins that upon contact cause a change in fluorescent signal from the clone, and wherein the effect of the presence of the biomolecule or bioactivity is to cause such contact.

55. (Currently Amended) The method of claim 54, wherein the substrate is for a thioesterase.